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TRIzol for plasmid DNA isolation

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▼TRIzol (Gibco BRL; US Patent No 5.346.994) is a well-accepted reagent for isolation of total RNA, chromosomal DNA, and proteins from tissues and cells (Ref. 1). We have found that its use may be extended to plasmid DNA isolation from transformed *Escherichia coli* cells. Most importantly, the method allows convenient performance a MIDI-preparation of plasmid DNA in a microcentrifuge tube. The yield of plasmid DNA isolated with TRIzol is not less than that obtained by alkaline lysis method (3–6 μg DNA per 1 ml overnight bacterial culture; Ref. 2) but the TRIzol-based isolation is faster than conventional methods of alkaline lysis or lysis by boiling (Ref. 3) for MIDI-preparation of plasmid DNA, and allows parallel experiments, if necessary. We have used this method for isolation of recombinant plasmids based on pGEM-5Z vector (Promega) amplified in DH5α and JM109 *E. coli* strains. The recovered DNA is pure enough for restriction analysis, generation of radiolabeled probes, manual sequencing and other enzymatic reactions.

Protocol

Grow a 50 ml culture of the bacterial strain carrying the plasmid overnight. Pellet cells by centrifugation. Lyse cells by adding 2 ml of TRIzol reagent (a larger volume of TRIzol does not substantially increase the yield) and shear the lysate by passing it three times through a 5 ml syringe fitted with a 21 gauge needle. There is no need to resuspend the bacterial pellet first. Transfer the lysate to two microcentrifuge tubes. Incubate the samples for 5 min at room temperature, then add 0.2 ml chloroform per 1 ml TRIzolused in the initial lysis, shake tubes, and continue the incubation for 3 min at room temperature. Centrifuge the samples in a microcentrifuge at maximum speed for 15 min at 4°C or room temperature and transfer the aqueous phase to fresh tubes. Add DNase-free RNase, to final concentration 25 μg/ml, and incubate for 30 min at 37°C. Add 0.5 ml isopropanol per 1 ml of TRIzol used for the initial lysis. Incubate samples at room temperature for 10 min and centrifuge at maximum speed in a microcentrifuge for 10 min at 4°C. Wash the DNA pellet once with 75% ethanol and centrifuge at maximum speed for 5 min. Air dry the pellet and redissolve the DNA in water. The plasmid DNA should have an absorbance ratio (260/280 nm) of 1.9–2.0, and be ready for restriction analysis (Fig. 1), radiolabeling and ligation.

The method allows processing for MIDI-preparation (up to 300 μg) in microcentrifuge tubes. It does not require any reagents other than those already found in most molecular biology laboratories. Preparation of special solutions or purchase of expensive kits is not needed. The procedure takes 90 min and does not require your complete attention. Finally, it gives a consistently good yield of pure plasmid DNA.

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References

1 Chomczynski, P. (1993) *BioTechniques* 15, 532–537.
2 Sambrook, J., Fritch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, 2nd edn*, Cold Spring Harbor Laboratory Press.
3 Holmes, D.S. and Quigley, M. (1981) *Anal. Biochem.* 114:, 193.

Products Used

TRIzol: TRIzol from Life Technologies (Gibco BRL)
TRIzol: TRIzol from Life Technologies (Gibco BRL)
pGEM-5Z vector: pGEM-5Z vector from Promega Corporation
NcoI: NcoI from Life Technologies (Gibco BRL)

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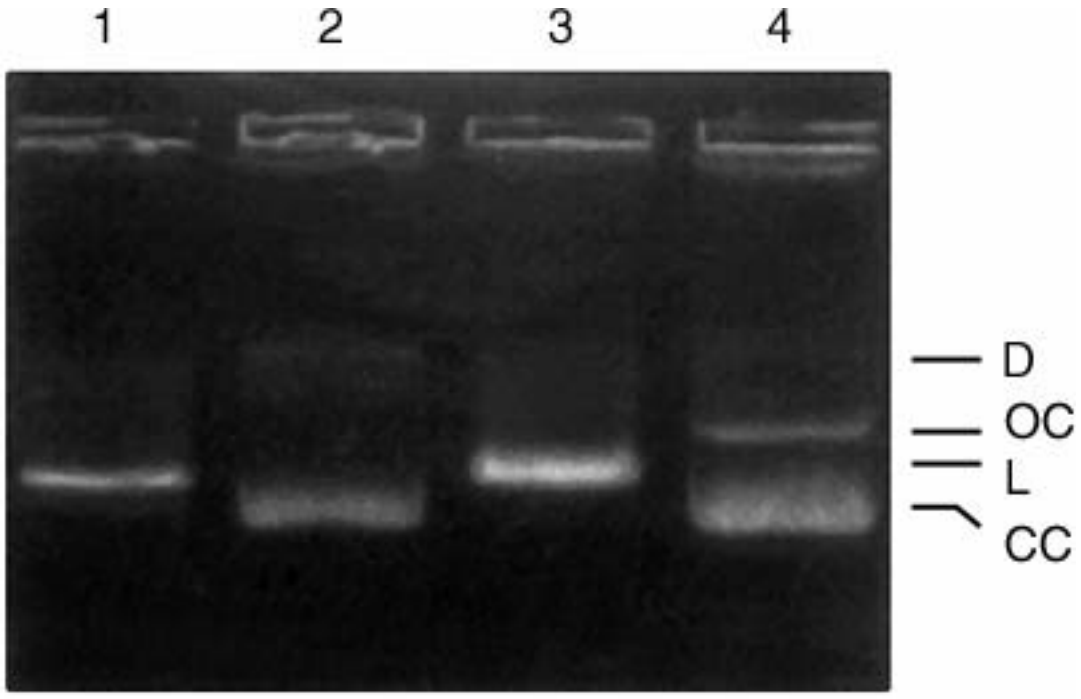

FIGURE 1. Comparative characterization of plasmid DNA by restriction analysis and electrophoresis in an 0.8% agarose gel. Lanes 2 and 4, native plasmids isolated by the TRIzol and alkaline lysis methods, respectively. Lanes 1 and 3, linear plasmids resulting from the restriction (TRIzol and alkaline lysis methods, respectively). D, dimeric plasmid; OC, open circular plasmid; L, linear plasmid; CC, covalently closed plasmid. Comparison of lanes 2 and 4 indicates that there is less or no open or circular form of the TRIzol-purified plasmid. Plasmid DNA (0.5 μg) was incubated with 1 U of restriction endonuclease NcoI (Gibco BRL) for 1 h at 37°C.